

**WHAT IS CLAIMED IS:**

1. A mutein recombinant tissue protective cytokine lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine comprising at least one responsive cellular protective activity selected from the group consisting of protecting, maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.
2. The recombinant tissue protective cytokine of claim 1, comprising one or more altered amino acid residue between position 11 to 15 of SEQ ID NO:10 [SEQ ID NO:1], position 44 to 51 of SEQ ID NO 10 [SEQ ID NO:2], position 100-108 of SEQ ID NO [SEQ ID NO:3], or position 146-151 of SEQ ID NO 10 [SEQ ID NO:4].
3. The recombinant tissue protective cytokine of claim 1, comprising an altered amino acid residue at one or more of the following positions of SEQ ID NO: 10: 7, 20, 21, 29, 33, 38, 42, 59, 63, 67, 70, 83, 96, 126, 142, 143, 152, 153, 155, 156, or 161.
4. The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with one or more of the amino acid residue substitutions of SEQ ID NOs: 15-105 and 119.
5. The recombinant tissue protective cytokine of claim 1, comprising the amino acid sequence of SEQ ID NO: 10 with a deletion of amino acid residues 44-49 of SEQ ID NO: 10.
6. The recombinant tissue protective cytokine of claim 1, comprising, the amino acid sequence of SEQ ID NO: 10 with at least one of the following amino acid residue substitutions of SEQ ID NOs: 106-118.
7. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, further comprising a chemical modification of one or more amino acids.
8. The recombinant tissue protective cytokine of claim 7, wherein the chemical modification comprises altering the charge of the recombinant tissue protective cytokine.

9. The recombinant tissue protective cytokine of claim 8, wherein a positive or negative charge is chemically added to an amino acid residue where a charged amino acid residue is modified to an uncharged residue.
10. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6,  
5 wherein said cytokine is a human erythropoietin mutein.
11. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6,  
wherein said cytokine is a human phenylglyoxal erythropoietin mutein.
12. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6,  
wherein the responsive mammalian cell comprises a neuronal, muscle, heart, lung, liver,  
10 kidney, small intestine, adrenal cortex, adrenal medulla, capillary, endothelial, testis, ovary, endometrial, or stem cell.
13. The recombinant tissue protective cytokine responsive mammalian cell of any one of claims 1, 2, 3, 4, 5, or 6, comprising a photoreceptor, ganglion, bipolar, horizontal, amacrine, Muieller, myocardium, pace maker, sinoatrial node, sinus node, atrioventricular  
15 node, bundle of His, hepatocyte, stellate, Kupffer, mesangial, goblet, intestinal gland, enteral endocrine, glomerulosa, fasciculate, reticularis, chromaffin, pericyte, Leydig, Sertoli, sperm, Graffian follicles, primordial follicles, endometrial stroma, and endometrial cell.
14. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6,  
20 wherein said cytokine is capable of traversing an endothelial cell barrier.
15. The recombinant tissue protective cytokine of claim 14, wherein the endothelial cell barrier comprises the blood-brain barrier, the blood-eye barrier, the blood testes barrier, the blood-ovary barrier, and the blood-uterus barrier.
16. The recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6,  
25 wherein said cytokine is selected from the group consisting of:
- i. a cytokine having a reduced number or no sialic acid moieties;
  - ii. a cytokine having a reduced number or no N-linked or O-linked carbohydrates;
  - iii. a cytokine having at least a reduced carbohydrate content by virtue of treatment

- of native cytokine with at least one glycosidase;
- iv. a cytokine having at least one or more oxidized carbohydrates;
- v. a cytokine having at least one or more oxidized carbohydrates and is chemically reduced;
- 5 vi. a cytokine having at least one or more modified arginine residues;
- vii. a cytokine having at least one or more modified lysine residues or a modification of the N-terminal amino group of a cytokine molecule;
- viii. a cytokine having at least a modified tyrosine residue;
- ix. a cytokine having at least a modified aspartic acid or glutamic acid residue;
- 10 x. a cytokine having at a modified tryptophan residue;
- xi. a cytokine having at least one amino acid group removed;
- xii. a cytokine having at least one opening of at least one of the cystine linkages in the cytokine molecule;
- xiii. a truncated cytokine;
- 15 xiv. a cytokine having at least one polyethylene glycol molecule attached;
- xv. a cytokine having at least one fatty acid attached;
- xvi. a cytokine having a non-mammalian glycosylation pattern by virtue of the expression of a recombinant cytokine in non-mammalian cells; and
- xvi. a cytokine having at least one histidine tagged amino acid to facilitate
- 20 purification.

17. The recombinant tissue protective cytokine of claim 16 wherein said cytokine is an asialoerythropoietin.
18. The recombinant tissue protective cytokine of claim 17, wherein said asialoerythropoietin is human asialoerythropoietin.
- 25 19. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is hyposialylated or hypersialylated.
20. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 sialic acid moieties.
21. The recombinant tissue protective cytokine of claim 16, wherein said cytokine
- 30 comprises more than the fourteen sialic acid moieties present in native erythropoietin.

22. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin with no N-linked carbohydrates.
23. The recombinant tissue protective cytokine of claim 22, wherein said cytokine is an erythropoietin with no O-linked carbohydrates.
- 5 24. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is treated with at least one glycosidase.
25. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is periodate-oxidized erythropoietin.
- 10 26. The recombinant tissue protective cytokine of claim 25, wherein said periodate-oxidized erythropoietin is chemically reduced with sodium cyanoborohydride.
27. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an R-glyoxal moiety on the one or more arginine residues, wherein R is aryl or alkyl moiety.
- 15 28. The recombinant tissue protective cytokine of claim 27, wherein said cytokine is phenylglyoxal-erythropoietin.
29. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is modified by reaction with a vicinal diketone selected from the group consisting of 2,3-butanedione and cyclohexanedione.
- 20 30. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is an erythropoietin in which an arginine residue is reacted with 3-deoxyglucosone.
31. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a molecule having at least one biotinylated lysine or N-terminal amino group.
32. The recombinant tissue protective cytokine of claim 16, wherein said cytokine is a glucitolyl lysine erythropoietin or fructosyl lysine erythropoietin.
- 25 33. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one carbamylated lysine residue.

34. The recombinant tissue protective cytokine of claim 33, wherein said carbamylated cytokine is comprised of alpha-N-carbamoylerythropoietin; N-epsilon-carbamoylerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylerythropoietin; alpha-N-carbamoylasialoerythropoietin; N-epsilon-carbamoylasialoerythropoietin; alpha-N-carbamoyl, N-epsilon-carbamoylasialoerythropoietin; alpha-N-carbamoylhyposialoerythropoietin; N-epsilon-carbamoylhyposialoerythropoietin; and alpha-N-carbamoyl, N-epsilon-carbamoylhyposialoerythropoietin.
35. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one acylated lysine residue.
36. The recombinant tissue protective cytokine of claim 35, wherein said cytokine comprises at least one acylated lysine residue.
37. The recombinant tissue protective cytokine of claim 36, wherein said cytokine comprises at least one acylated lysine residue.
38. The recombinant tissue protective cytokine of claim 37, wherein a said acetylated cytokine is comprised of alpha-N-acetylerythropoietin; N-epsilon-acetylerythropoietin; alpha-N-acetyl, N-epsilon-acetylerythropoietin; alpha-N-acetylasialoerythropoietin; N-epsilon-acetylasialoerythropoietin; alpha-N-acetyl, N-epsilon-acetylasialoerythropoietin; alpha-N-acetylhyposialoerythropoietin; N-epsilon-acetylhyposialoerythropoietin; and alpha-N-acetyl, N-epsilon-acetylhyposialoerythropoietin.
39. The recombinant tissue protective cytokine of claim 35, wherein a lysine residue of said cytokine is succinylated.
40. The recombinant tissue protective cytokine of claim 39, wherein said succinylated cytokine is comprised of alpha-N-succinylerythropoietin; N-epsilon-succinylerythropoietin; alpha-N-succinyl, N-epsilon-succinylerythropoietin; alpha-N-succinylasialoerythropoietin; N-epsilon-succinylasialoerythropoietin; alpha-N-succinyl, N-epsilon-succinylasialoerythropoietin; alpha-N-succinylhyposialoerythropoietin; N-epsilon-succinylhyposialoerythropoietin; and alpha-N-succinyl, N-epsilon-succinylhyposialoerythropoietin.

41. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises at least one lysine residue modified by 2, 4, 6 trinitrobenzenesulfonate sodium or another salt thereof.
42. The recombinant tissue protective cytokine of claim 16, wherein said cytokine  
5 comprises at least one nitrated or iodinated tyrosine residue.
43. The recombinant tissue protective cytokine of claim 16, wherein said cytokine comprises an aspartic acid or glutamic acid residue that is reacted with a carbodiimide followed by reaction with an amine.
44. The recombinant tissue protective cytokine of claim 16, wherein a said amine is  
10 glycynamide.
45. An isolated nucleic acid molecule that comprises a nucleotide sequence which encodes a polypeptide comprising the recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6.
46. A vector comprising a nucleic acid molecule of claim 45.
- 15 47. An expression vector comprising a nucleic acid molecule of claim 45 and at least one regulatory region operably linked to the nucleic acid molecule.
48. The vector of claim 46 or 47 that is a pCiNeo vector.
49. A genetically-engineered cell which comprises a nucleic acid molecule of claim 45.
50. A cell comprising the expression vector of claim 45.
- 20 51. A pharmaceutical composition comprising a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes, the cytokine having at least one responsive cellular protective activity selected from the group consisting of protecting,  
25 maintaining, enhancing or restoring the function or viability of a responsive mammalian cell, tissue or organ.

52. The pharmaceutical composition of claim 51, formulated for oral, intranasal, or parenteral administration.
53. The pharmaceutical composition of claim 51, formulated as a perfusate solution.
54. A method for protecting, maintaining or enhancing the viability of a cell, tissue or  
5 organ isolated from a mammalian body comprising exposing said cell, tissue or organ to a  
pharmaceutical composition comprising a mutein recombinant tissue protective cytokine.
55. The method of claim 54, wherein the protection does not effect bone marrow.
56. A method for protecting, maintaining or enhancing the viability of a cell, tissue or  
organ isolated from a mammalian body comprising exposing said cell, tissue or organ to a  
10 pharmaceutical composition comprising a recombinant tissue protective cytokine of any one  
of claims 1, 2, 3, 4, 5, or 6, that lacks at least one activity selected from the group consisting  
of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity  
and increasing production of thrombocytes.
57. Use of a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or  
15 6, that lacks at least one activity selected from the group consisting of increasing  
hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and  
increasing production of thrombocytes, for the preparation of a pharmaceutical composition  
for the protection against and prevention of a tissue injury as well as the restoration of and  
rejuvenation of tissue and tissue function in a mammal.
- 20 58. The use of claim 57, wherein the injury is caused by a seizure disorder, multiple  
sclerosis, stroke, hypotension, cardiac arrest, ischemia, myocardial infarction, inflammation,  
age-related loss of cognitive function, radiation damage, cerebral palsy, neurodegenerative  
disease, Alzheimer's disease, Parkinson's disease, Leigh disease, AIDS dementia, memory  
loss, amyotrophic lateral sclerosis, alcoholism, mood disorder, anxiety disorder, attention  
25 deficit disorder, autism, Creutzfeld-Jakob disease, brain or spinal cord trauma or ischemia,  
heart-lung bypass, chronic heart failure, macular degeneration, diabetic neuropathy, diabetic  
retinopathy, glaucoma, retinal ischemia, or retinal trauma.
59. A method for facilitating the transcytosis of a molecule across an endothelial cell  
barrier in a mammal comprising administration to said mammal a composition comprising

said molecule in association with a recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, increasing blood pressure, hyperactivating platelets, and increasing production of thrombocytes.

- 5     60.     The method of claim 59, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

61.     The method of claim 59, wherein said endothelial cell barrier is selected from the group consisting of the blood-brain barrier, the blood-eye barrier, the blood-testis barrier, the blood-ovary barrier, the blood-heart barrier, the blood-kidney barrier, and the blood-placenta barrier.
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62.     The method of claim 59, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, an antiviral agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.

- 15     63.     A composition for transporting a molecule via transcytosis across an endothelial cell barrier comprising said molecule in association with a recombinant tissue protective cytokine, of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activity and increasing production of thrombocytes.

- 20     64.     The composition of claim 63, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

65.     The composition of claim 63, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense oligonucleotide, an antibody, an immunosuppressant, a dye, a marker, or an anti-cancer drug.
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66.     Use of an recombinant tissue protective cytokine of any one of claims 1, 2, 3, 4, 5, or 6, lacking at least one activity selected from the group consisting of increasing hematocrit, vasoactive action, hyperactivating platelets, pro-coagulant activities and increasing production of thrombocytes.



67. The use of claim 66, wherein said association is a labile covalent bond, a stable covalent bond, or a non-covalent association with a binding site for said molecule.

68. The use of claim 66, wherein said molecule is a receptor agonist or antagonist hormone, a neurotrophic factor, an antimicrobial agent, a radiopharmaceutical, an antisense  
5 oligonucleotide, an antibody, an immunosuppressant, a dye, or a marker, or an anti-cancer drug.